Stories Behind the Cocktail and Synergism

Ting-Chao Chou, Ph.D.
Founder & President, PD Science, LLC USA

5th World Congress on Virology *(Virology-2015)*
December 7-9, 2015 Atlanta, GA USA
Opening Keynote Forum, December 7, 2015, 9:00-9:45AM

RESEARCHERID

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Chou, Ting-Chao

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ResearcherID: B-4111-2009

Other Names: T.C. Chou; TC Chou

URL: http://www.researcherid.com/rid/B-4111-2009

My Institutions (more details)

Primary Institution: Memorial Sloan-Kettering Cancer Center

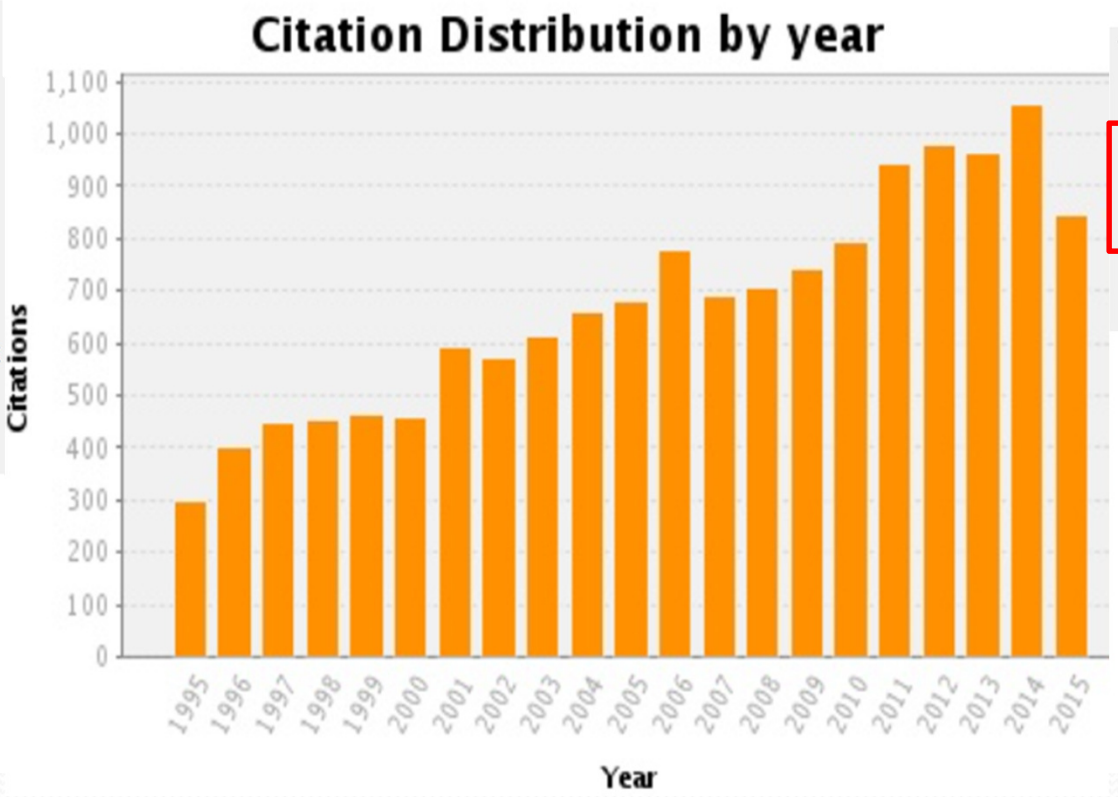
Sub-org/Dept:

Role: Researcher (Academic)

My Publications

My Publications (273)
View Publications
Citation Metrics

ResearcherID labs
Create A Badge
Collaboration Network
Citing Articles Network



Total Articles in Publication List: **273**

Articles With Citation Data: **259**

Sum of the Times Cited: **16972**

Average Citations per Article: **65.53**

h-index: **58**

Last Updated: **11/16/2015 17:33 GMT**

Synergism is more than Additive Effect

Antagonism is less than Additive Effect

What is "Additive Effect"?

A Quiz:

If I_1 and I_2 each inhibits 30% of a bio-system, then in combination, if additive (i.e., no synergism nor antagonism) should inhibit:

A. 60% [30+30=60] (Sum)

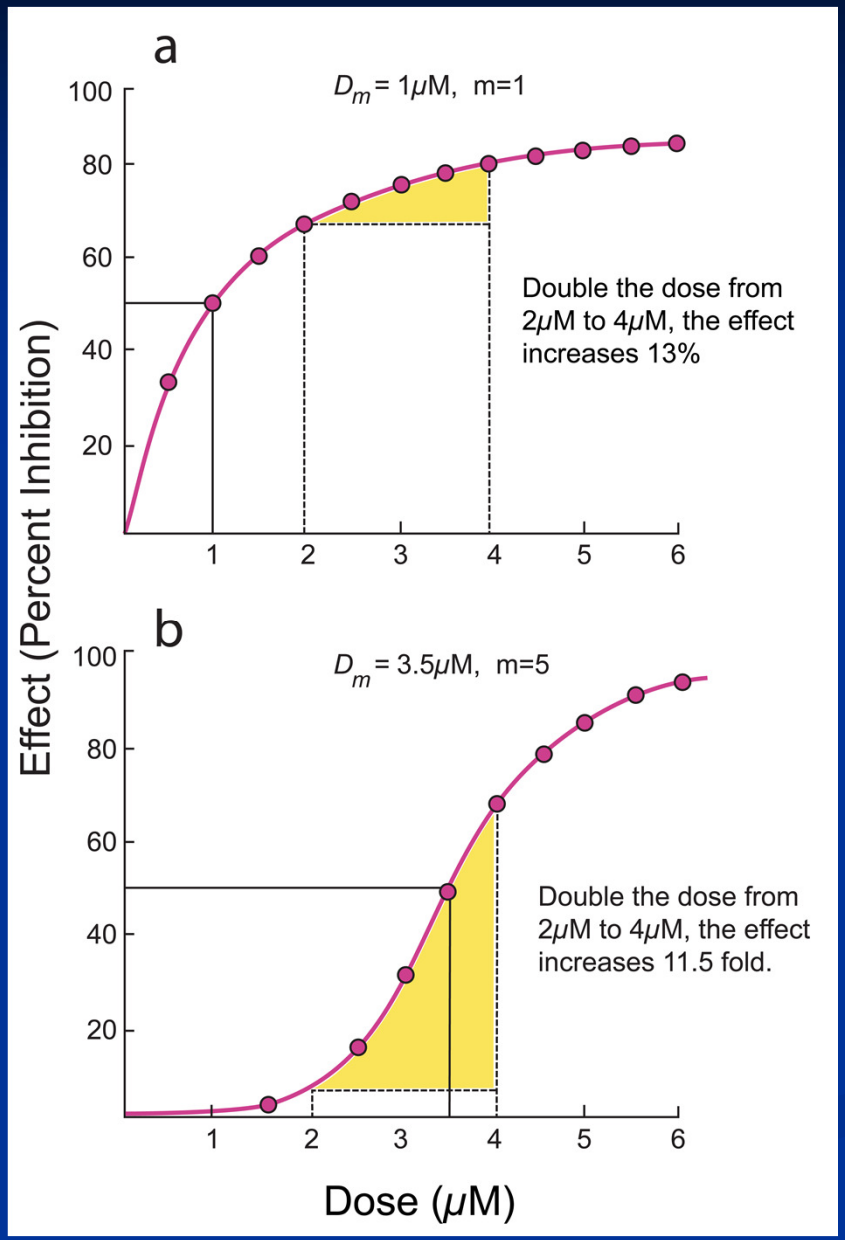
B. <60% [60+60=120 , Sum >100% ?!]

C. 51% (1-0.3)x(1-0.3)=0.49, [1-0.49=0.51]

D. None of the above

E. Don't know

Why Determination of Synergism is More Complicated than Expected:



An Example for Illustration :

We need both
"**Potency**" (D_m) and "**Shape**" (m)

To Analyze Pharmaco-*Dynamics*

[13% vs 11.5 Fold]

"a" + "b" What is Additive ?

What is Synergy?

Don't Know** without a Dynamic **Theory !

Why Drug Combination?

- **"Synergy"** Increases Efficacy
- **"Synergy"** Decreases Toxicity

> **Improve Therapeutic Index**

- Minimize and/or Delay **Drug Resistance**
- Attack **Multiple Targets**
- Attack Different **Stages of** Life Cycle
- An Inactive Compound may Induced **"Potentiation"**
- May Improve **Pharmacokinetics of Another** in Addition to **Pharmacodynamics**

"Synergy Definition" for Drug Combination *(Controversy for Over 100 Years)*

- **Drug Combination: Widely Used in Cancer , AIDS & Chinese Medicine**
- **Synergism: 20 Different Synergy Definitions**
  None Supports the Others ! A Total Mess !!
- **Combination Index Theorem: Chou & Talalay, 1984, Quantitative Determines**

CI <1, Synergism; CI=1, Additive Effect; CI>1, Antagonism

Challenge: ***If No Clear Definition for Synergy: NIH, FDA, Patent Office and Journal Editors have No Basis to Judge or to Regulate the "Synergy Claims"***

— The Dispel of the Century-Old Controversy of "Synergy Claims"

Chou & Talalay: The CI Method Prevailed. The Most Cited Method *of All Time*

- *No.1: Adv. Enz. Regul. 22:27-55, 1984 (Created the CI theory) (Cited 4,450 times by 693 journals)*
- *No.2: Pharmacol. Rev. 58:621-681, 2006 (Review , 61-pages) (Cited 1,408 times by 404 journals)*
- *No.7: Cancer Res. 70: 440-446, 2010 (Perspectives) (Cited 782 times in 237 journals in 5 yrs)*

Trends of Drug Combination Methods for Synergy Determination, 1900-2015*

Method, Main Theory and Reference Source	Thomson Reuters Web of Science Citation DataBase: Trend of Citation				Total Citations Since Publication	Average Citations per year
	2011	2012	2013	2014		
A. Chou, TC & Talalay, P Adv. Eng. Regul. 1984; 22:27-55	289	257	283	**325**	**3,597**	**116.0**
B. Chou, TC Pharmacol. Rev. 2006;58: 621-681	119	146	158	**205**	**1,016**	**112.9**
C. Berenbaum, MC Pharmacol. Rev. 1989; 41: 93-141	42	37	46	42	**912**	33.8
D. Bliss, CI Ann. Appl. Biol. 1939; 26: 585-615	48	70	68	76	**705**	9.2
E. Steel GG & Peekham MJ Int. J. Radiant. Oncol. BioPhys. 1979; 5: 85-91	16	22	14	15	680	18.4
F. Greco, WR et al Pharamacol. Rev. 1995; 47: 331-385	33	38	56	56	**614**	29.2
G. Chou TC Cancer Res. 2010; 70: 440-446	41	75	123	**174**	**582**	**116.4**
H. Elion GB, Singer S & Hitchings GH J. Biol. Chem. 1954; 208: 477-488	3	9	4	4	**449**	7.2
I. Tallarida, RJ J. Pharmacol. Exp. Ther. 2001; 298: 865-872	26	26	34	34	**353**	23.5
J. Prichard, MN & Shipman C Jr Antiviral Res. 1990; 14: 181-205	16	18	16	24	**342**	13.2
K. Webb J.L. Acad. Press. 1963; 1: 66-79, 488-512	8	9	12	6	**#262**	5.1
L. Loewe, S Pharmacol. Rev. 1957; 9: 237-242	3	0	4	5	**118**	2.0

*Based on Thomson Reuters Web of Science **All** Database Collection, as of June 3, 2015. (Updated 12.4.2015)

#Based on Google Scholar Citations, as of June 3, 2015.



Change photo

Ting-Chao Chou – Google Scholar Citations

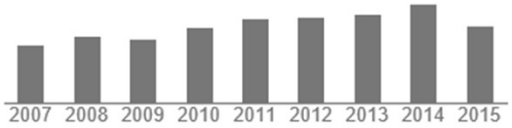
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Title	+ Add	More	1–20	Cited by	Year
Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors TC Chou, P Talalay Advances in enzyme regulation 22, 27-55				4431	1984
Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies TC Chou Pharmacological reviews 58 (3), 621-681				1399	2006
Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian–human immunodeficiency virus infection TW Baba, V Liska, R Hofmann-Lehmann, J Vlasak, W Xu, S Ayehunie, ... Nature medicine 6 (2), 200-206				834	2000
Drug combination studies and their synergy quantification using the Chou-Talalay method TC Chou Cancer research 70 (2), 440-446				772	2010
Computerized quantitation of synergism and antagonism of taxol, topotecan, and cisplatin against human teratocarcinoma cell growth: a rational approach to clinical protocol design TC Chou, RJ Motzer, Y Tong, GJ Bosl Journal of the National Cancer Institute 86 (20), 1517-1524				438	1994
Analysis of combined drug effects: a new look at a very old problem TC Chou, P Talalay Trends in Pharmacological Sciences 4, 450-454				380	1983

Google Scholar

Citation indices	All	Since 2010
Citations	22888	8473
h-index	65	32
i10-index	239	120



Add co-authors

- Jon T. Njardarson
- Chung K Chu
- Anyi Li
- Jonathan Zager
- Chiu-Chung Young
- Dennis P. Curran
- Lorenza Gandola
- Lusheng Wang
- Chee Keong Chen
- Nilendu Gupta

Co-authors Edit...

Yuman Fong

The Mass-Action Law Based Theory and Computerized Simulation

- The Mass-Action Law Based General Equations

  The Median-Effect Eq. (MEE) *[The Unified Theory]*

  [Chou TC, J. Theor. Biol. 59: 253-276, 1976] (Cited 287 times)

  The Combination Index Eq. (CIE)

  [Chou TC & Talalay P. Adv Enz Regul 22: 27-55, 1984] (cited 4,450 times)

  [Chou TC. Pharmacol Rev. 58: 621-681, 2006] (Cited 1,408 times)

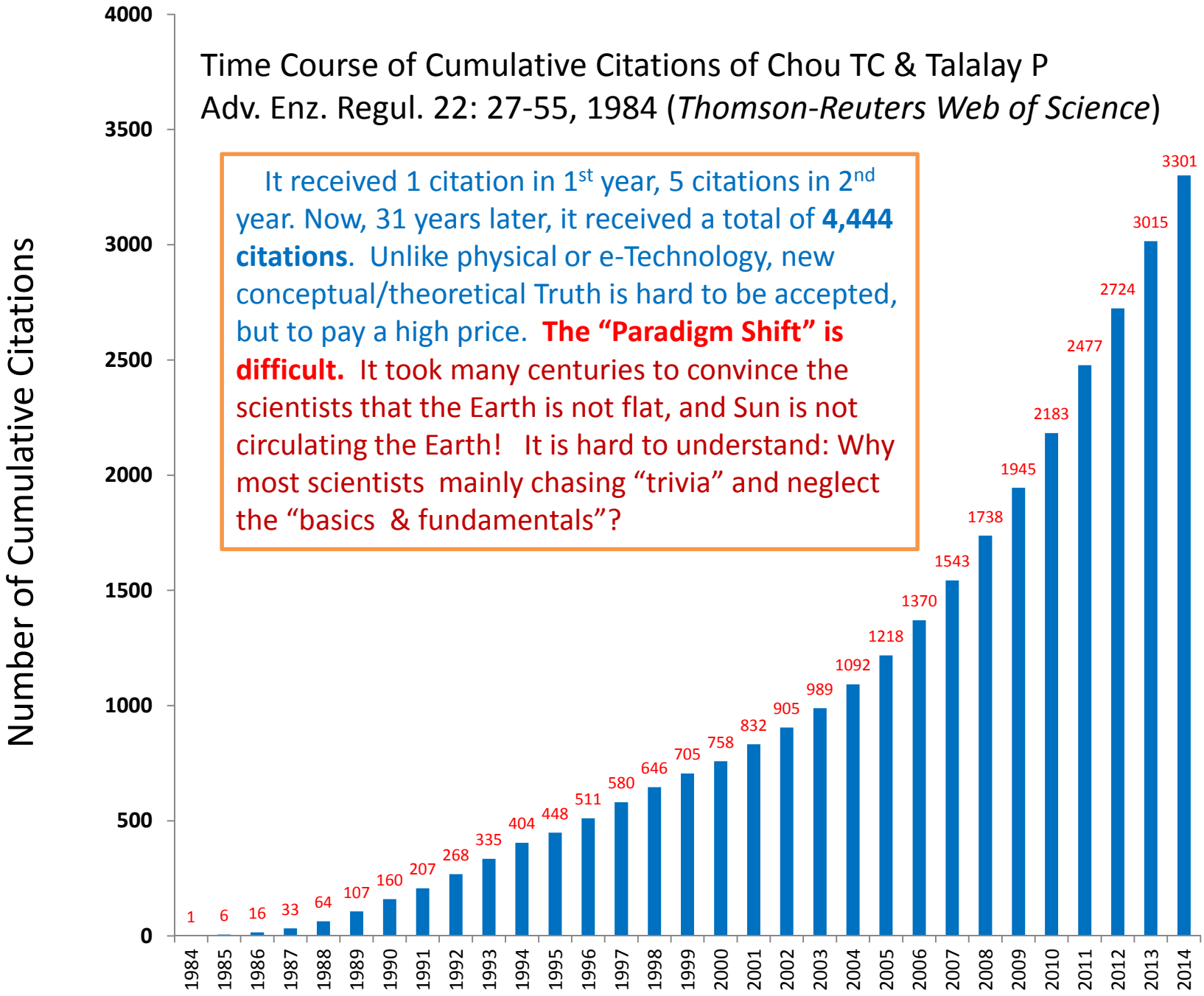
- Algorithms & Computer Simulation

  CI<1 (Synergism); CI=1 (Additive); CI >1 (Antagonism)

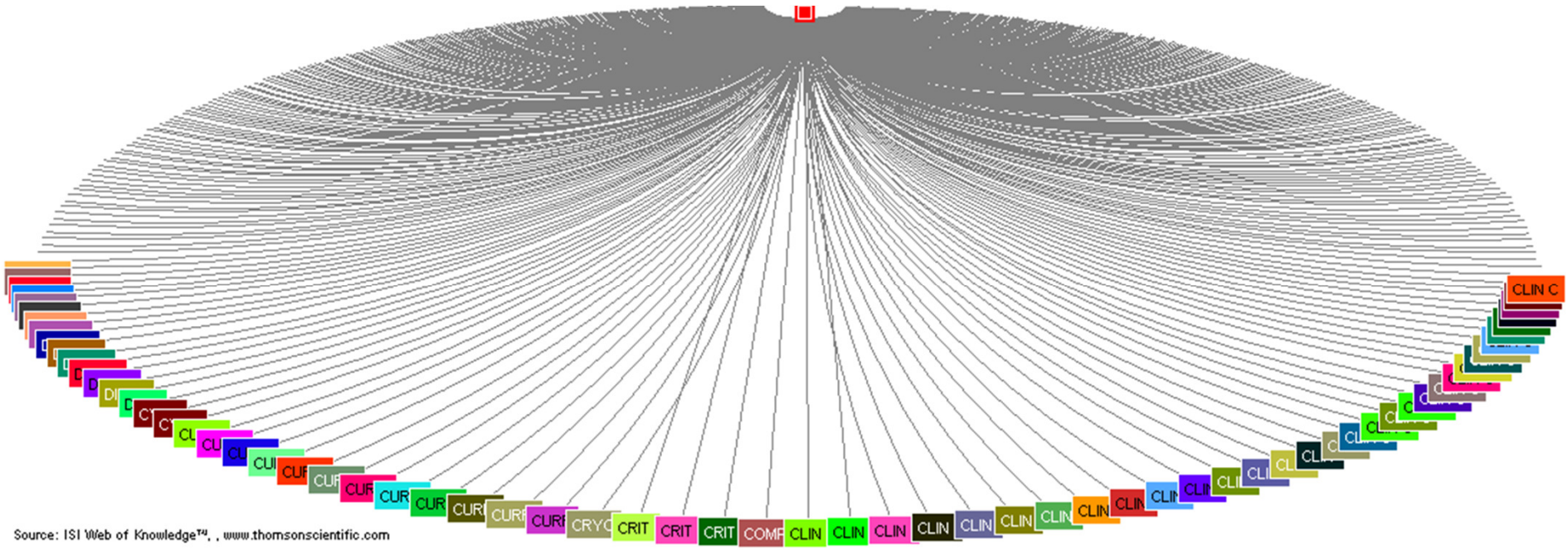
  Dose-Effect Dynamics *[Chou J. & Chou TC ; Biosoft, Cambridge UK 1983]*

  CalcuSyn Software *[Chou TC & Hayball M; Biosoft, Cambridge UK 1998]*

  CompuSyn Software *[Chou TC & Martin N; Combosyn, NJ USA 2005]*


Time Course of Cumulative Citations of Chou TC & Talalay P
Adv. Enz. Regul. 22: 27-55, 1984 (*Thomson-Reuters Web of Science*)
It received 1 citation in 1st year, 5 citations in 2nd year. Now, 31 years later, it received a total of **4,444 citations**. Unlike physical or e-Technology, new conceptual/theoretical Truth is hard to be accepted, but to pay a high price. **The "Paradigm Shift" is difficult.** It took many centuries to convince the scientists that the Earth is not flat, and Sun is not circulating the Earth! It is hard to understand: Why most scientists mainly chasing "trivia" and neglect the "basics & fundamentals"?
Number of Cumulative Citations
0
500
1000
1500
2000
2500
3000
3500
4000
1 6 16 33 64 107 160 207 268 335 404 448 511 580 646 705 758 832 905 989 1092 1218 1370 1543 1738 1945 2183 2477 2724 3015 3301
1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014

Quantitative-Analysis of Dose-Effect Relationships-
The Combined Effects of Multiple-Drugs or Enzyme-Inhibitors
[The Combination Index Theorem]



CHOU, TC; TALALAY, P

ADVANCES IN ENZYME REGULATION, 22: 27-55, 1984

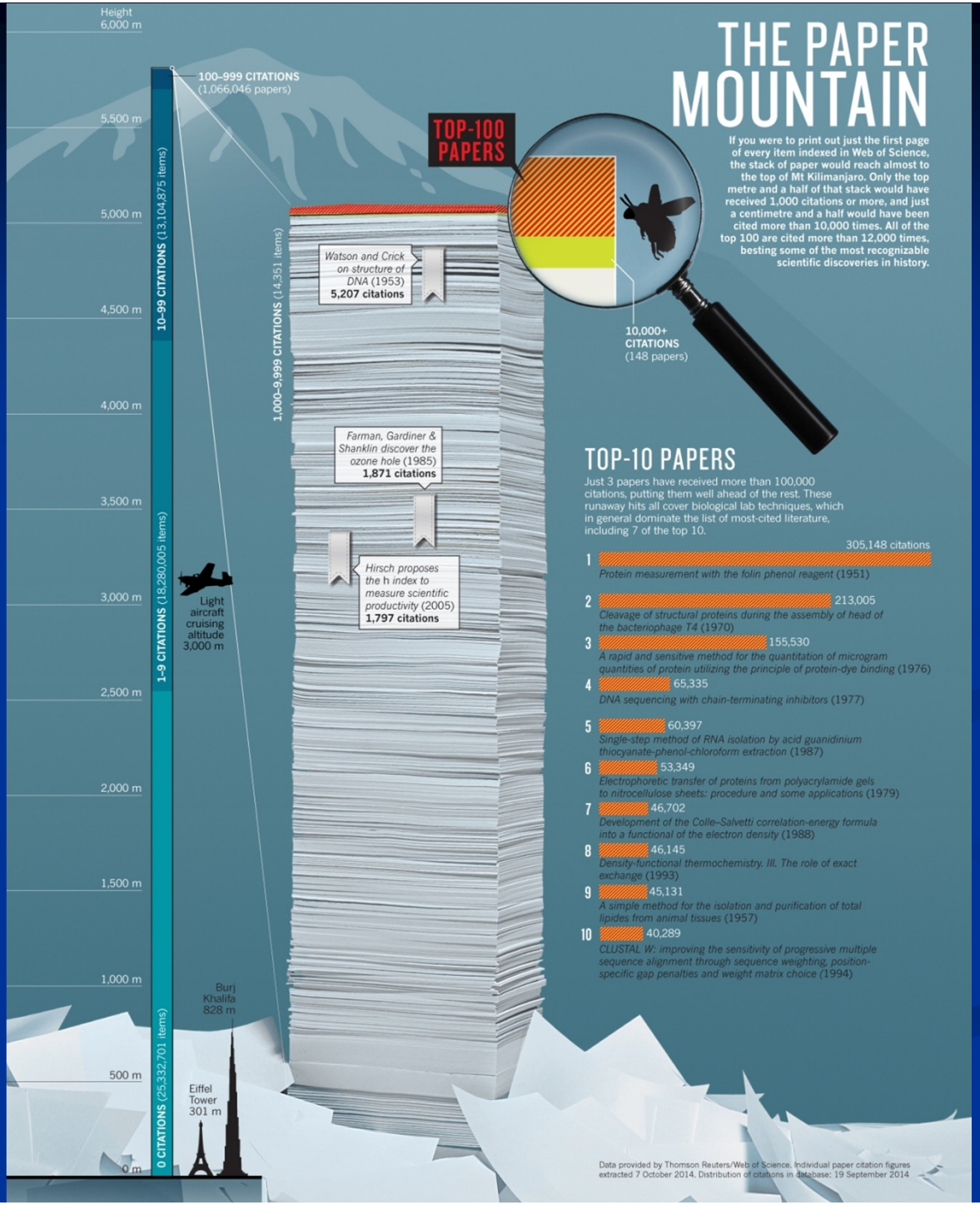
Times Cited: 4,444 (3,597 *In* 693 Journals*)

(*Among The Most Broadly Cited *Theoretical Papers* by Journals *of All Time*)

Science & Kilimanjaro

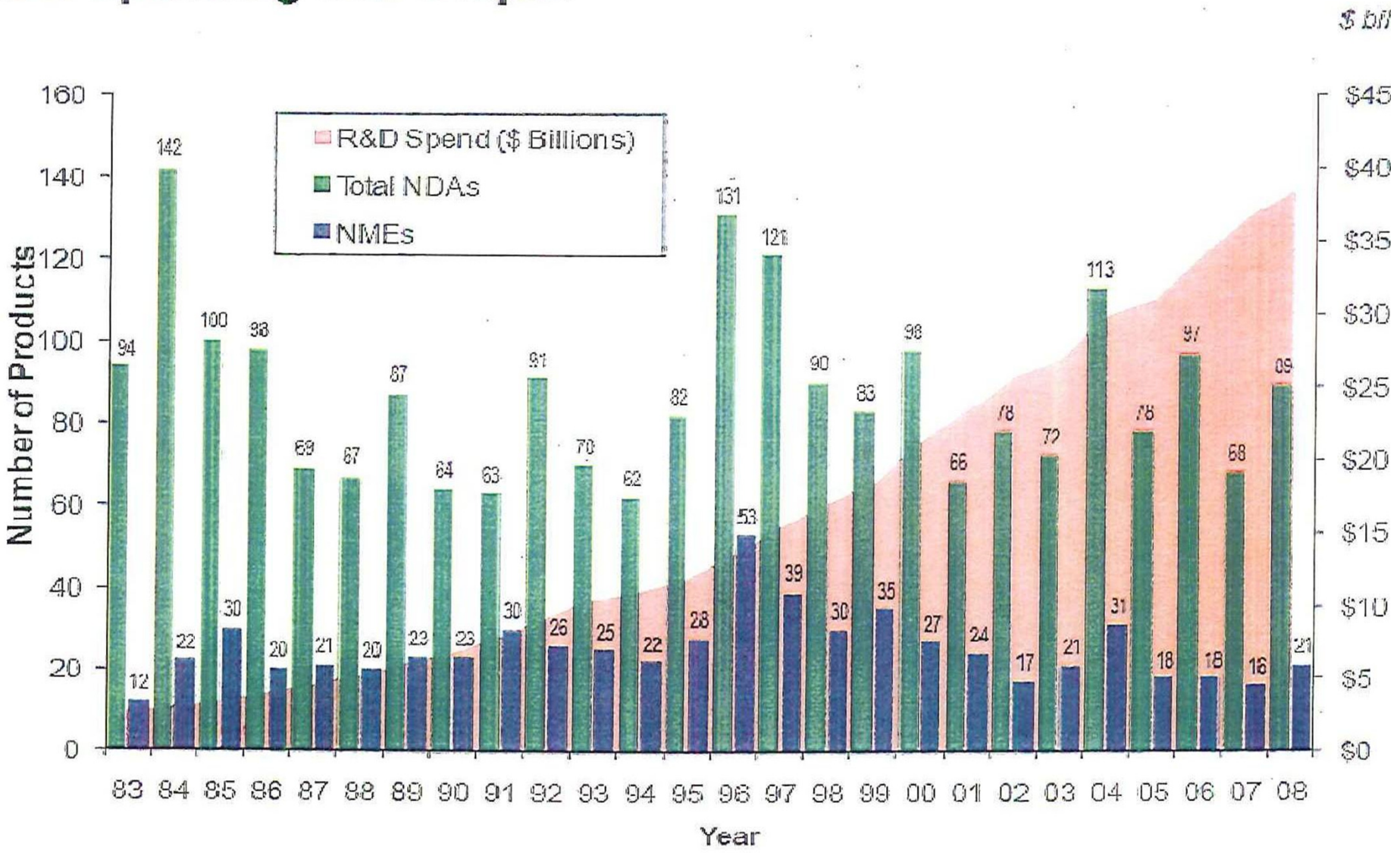
Based on the *Thomson Reuters Web of Science database,* and an article in *Nature 514: 550-553 (Oct.30, 2014),* there are 58 million scientific papers published during 1900-2014. The front pages of these papers can pile up to 5,800 meters, which is about the same height as Mt. Kilimanjaro, the highest peak in Africa (or equivalent to the height of 15 Empire State Buildings in New York piled-up all together). About 90% of the 100 highest cited papers are specific methodological, statistical or material science papers. There are very few theoretical or conceptual papers among them.

The general "theoretical article" of the "Mass-Action Law" published in 1984 for the "Combination Index Theorem" (Chou TC & Talalay P, *Advances in Enzyme Regulation, 22: 27-55, 1984*) has been cited by 3,597 papers (in over 693 different bio-medical journals), [Thomson Reuters Web of Science: *http://www.reseracherid.com/rid/B-4111-2009*]. This is equivalent to placing it on the "top 15 centimeter" on the peak of Mt. Kilimanjaro. This citation number is higher than those of 90% of Nobel laureates. In science, Nobel prize emphasizes breakthrough in innovation or discoveries, whereas Citation record reflects long lasting influence and impacts of the scientific work.


THE PAPER MOUNTAIN
If you were to print out just the first page of every item indexed in Web of Science, the stack of paper would reach almost to the top of Mt Kilimanjaro. Only the top metre and a half of that stack would have received 1,000 citations or more, and just a centimetre and a half would have been cited more than 10,000 times. All of the top 100 are cited more than 12,000 times, besting some of the most recognizable scientific discoveries in history.
Height
6,000 m
5,500 m
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4,500 m
4,000 m
3,500 m
3,000 m
2,500 m
2,000 m
1,500 m
1,000 m
500 m
0 m
100–999 CITATIONS (1,066,046 papers)
10–99 CITATIONS (13,104,875 items)
1–9 CITATIONS (18,280,005 items)
0 CITATIONS (25,332,701 items)
1,000–9,999 CITATIONS (14,351 items)
TOP-100 PAPERS
10,000+ CITATIONS (148 papers)
Watson and Crick on structure of DNA (1953) 5,207 citations
Farman, Gardiner & Shanklin discover the ozone hole (1985) 1,871 citations
Hirsch proposes the h index to measure scientific productivity (2005) 1,797 citations
Light aircraft cruising altitude 3,000 m
Burj Khalifa 828 m
Eiffel Tower 301 m
TOP-10 PAPERS
Just 3 papers have received more than 100,000 citations, putting them well ahead of the rest. These runaway hits all cover biological lab techniques, which in general dominate the list of most-cited literature, including 7 of the top 10.
305,148 citations
1 Protein measurement with the folin phenol reagent (1951)
2 213,005 Cleavage of structural proteins during the assembly of head of the bacteriophage T4 (1970)
3 155,530 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding (1976)
4 65,335 DNA sequencing with chain-terminating inhibitors (1977)
5 60,397 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction (1987)
6 53,349 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications (1979)
7 46,702 Development of the Colle–Salvetti correlation-energy formula into a functional of the electron density (1988)
8 46,145 Density-functional thermochemistry. III. The role of exact exchange (1993)
9 45,131 A simple method for the isolation and purification of total lipides from animal tissues (1957)
10 40,289 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice (1994)
Data provided by Thomson Reuters/Web of Science. Individual paper citation figures extracted 7 October 2014. Distribution of citations in database: 19 September 2014

Nearly Two Decades of Futility…

R&D Spending and Output



Ed Saltzman – Defined Health 2010

Strategies for Anti-Viral Drug Combination Therapies

I. "Quantification" of Synergy

– Computer Simulation of CI

II. "Econo-Green" Bio-Research

– Algorithm based Small Size Experimentation

III. "Efficient" Cocktail Design

– CI Directed Polygonogram

IV. Attacking Different "Stages of Life Cycle"

– Drugs With Different Mechanisms

Chou TC. *Pharmacol. Rev. 58: 621-681, 2006 (1,396 Citations in 404 journals in 9 years)*
– Free web access: http://pharmrev.aspetjournals.org/cgi/reprint/58/3/621

Scientific Basis of Anti-HIV "Cocktails"

- How to Design the Combination Cocktails ?
  [Polygonogram]
- Where Is the Theoretical Basis ?
  [MEE, CIE Dynamics]
- What Are the Conclusions ?
  [Quantitative Computer Simulations & Graphics]
  [Qualitative Projections of Possible Outcomes]
  [Reduction of the Number of Required Experimentations]

CI<1 (Synergism); CI=1 (Additive); CI >1 (Antagonism)

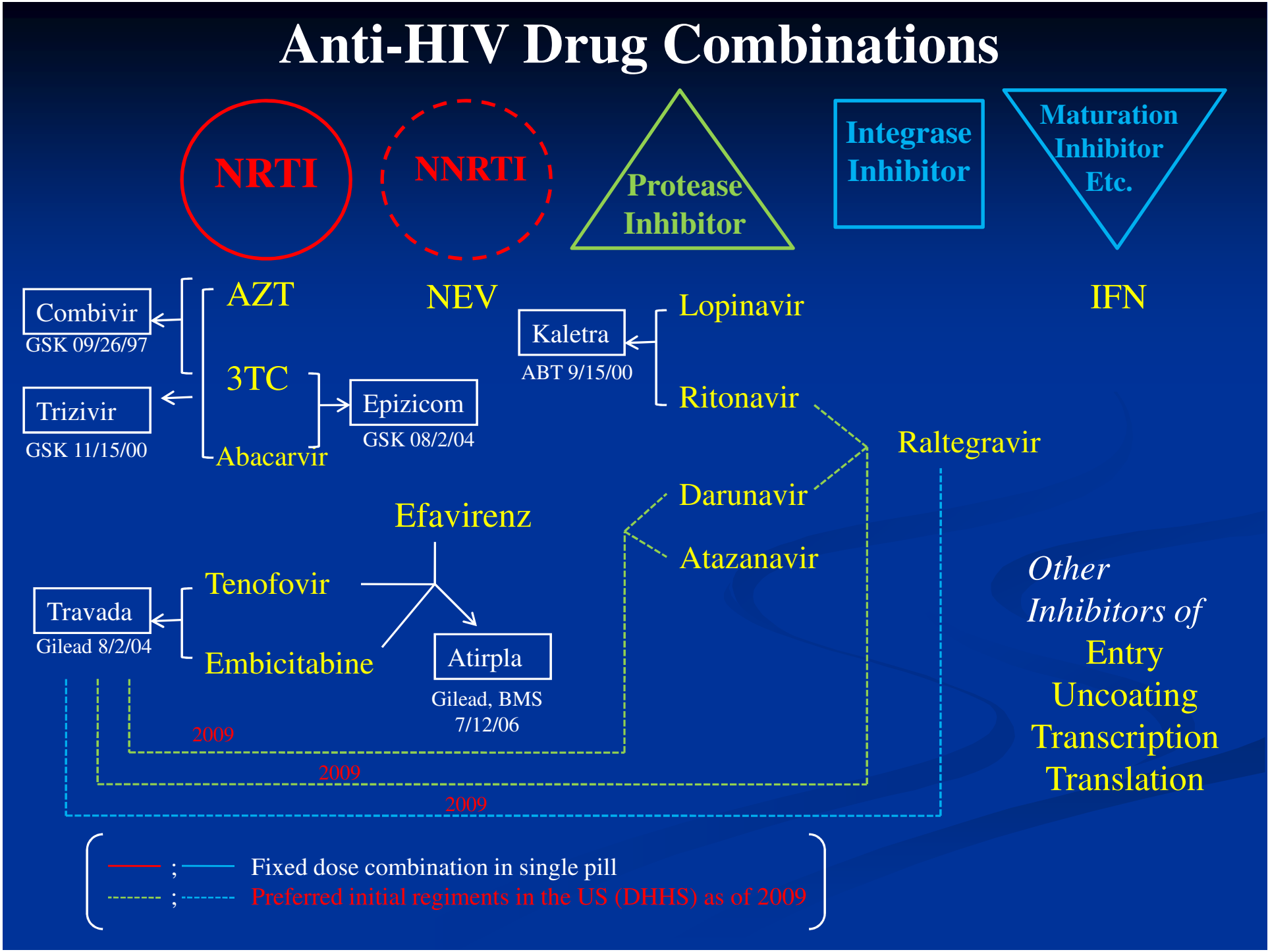
Anti-HIV Agents

Abbreviation	Drug's Name	Firm	Mode of Action
AZT	Azidothymidine Zidovudine, ZVD	GSK	NRTI
DDC	Dideoxyctidine Zalcitabine, HIVID	Roche	NRTI
DDI	Dideoxyinosine Didanosine, Videx	BMS	NRTI
D4T	Didehydro-dideoxy thymidine Stavudine, Zerit	BMS	NRTI
3TC	Lamivudine	GSK	NRTI
Emtriva	Emtricitabine,	Gilead	NRTI
Viread	Tenofovia	Gilead	NRTI
EFV	Efavirenz, Sustiva	BMS, Merck	NNRTI
ABC	Abacavir, Ziagen	Viir Healthcare, GSK	NRTI
NEV	Nevirapine BIRG-587, Viramune	Boehringer Ingelheim	NNRTI

Anti-HIV Agents (Cont'd)

Abbreviation	Drug's Name	Firm	Mode of Action
VX-478	Amprenavir	Vertex, GSK	PI
Ritonavir	ABT-84538, Norvir	Abbott	PI
SAQ	Saquinavir, SQV RO-318959	Roche	PI
IND	Indinavir, IDV MK-639, Crixivan	Merck	PI
VRC	AG1343, Viracept Nelfinavir	Agouron	PI
Lopinarir	ABT-378	Gilead	PI
Reyataz	Atazanavir	BMS	PI
Prezita	Darunavir	Maltum	PI
IFN	Interferon-α-2a Roferon-A	Roche	Maturation Inhibitor

Anti-HIV Drug Combinations



The Tales of Two Anti-HIV Clinical Trials

	AZT + 3TC	AZT + INF_α
Authors	J.J. Eron et al. (9 authors + Northern Am. HIV Working Party	D. Mildvan et al. (21 authors)
Publication	N. Engl. J. Med. 333: 1662-1669, 1995	Antiviral Therapy 1(2): 77-88, 1996
Journal Impact Factor	**28.5**	**3.1**
Number of Patients	**366** [Wrong Design & Wrong Data Analysis]	**36**
Surrogate Marker	CD_4^+, HIV-RNA	P24 Antigen, CD_4^+
Treatment Design	Fractionated Repeated Doses AZT Single Dose, 3TC 2 Doses	Fractionated Repeated Doses Both Drugs have 3 Doses
What They Have Proved	"Combination Effect is Greater than Each Drug Alone" **A+B > A or A+B > B** ($p<0.001$) This Does Not Need a Proof Not Possible to Claim Synergism	"Quantitative Determination of Synergism Using Combination Index Method" (CI < 1 indicate synergism) Use Chou-Talalay Method. *Adv. Enz. Regul. 22: 27-55, 1984*

Conclusion: Synergy is Not determined by *p* values but rather with the CI values
Synergy is Not a Statistical Issue but rather a Mass-Action Law Issue

The Unified Theory for "Econo-Green" Bio-Research

[40 Years of Work] -A Flow Chart

A. The Mass-Action Law

- *The Basics of Pharmacodynamics (PD) [>300 Equations]*
- *The Source of Bio-Informatics (BI)*

B. Mathematical Induction and Deduction

- *A Powerful Tool for Generalization*
- *Convergence Logics rather than Divergence Logics*

A+B. → The "Unified Theory"

Theory as the Common Denominator of Drug R&D

- The Median-Effect Equation of the Mass-Action Law

*(Chou TC. **1976**)*

- The Combination Index Equation (*Chou TC & Talalay P, **1984***)

↓

"Econo-Green Bio-Research" (*Chou TC. Integrative Biology, **2011***)

for Biomedical Research using Fewer Data Points with Computer Simulation

Median Effect Equation

(T.C. Chou, J Theor Biol 59: 253-276, 1976) [Cited 287 times]

HYPERBOLIC (First Order Or MICHAELIS-MENTEN), m = 1,
SIGMOIDAL (Higher Order), m>1
FOR BOTH CASES:

$$\frac{\mathbf{fa}}{\mathbf{fu}} = \left[\frac{\mathbf{D}}{\mathbf{Dm}}\right]^{m} \quad \{for\ General\ Pharmaco\text{-}Dynamics\}$$

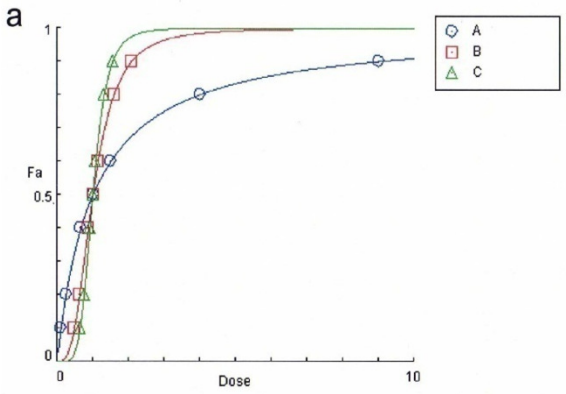
- **fa** = fraction of system affected, e.g. fractional inhibition
- **fu** = (1-fa) = fraction unaffected
- **D** = dose required to produce fa
- **Dm** = dose required to produce median effect. i.e. ED_{50}, IC_{50}
- ***m*** = sigmoidicity (shape), dynamic order

The Median-Effect Plot (The Chou Plot): Curves for Straight-lines:
Log (fa/fu) = m Log D - m Log Dm; ($y = ax + b$)

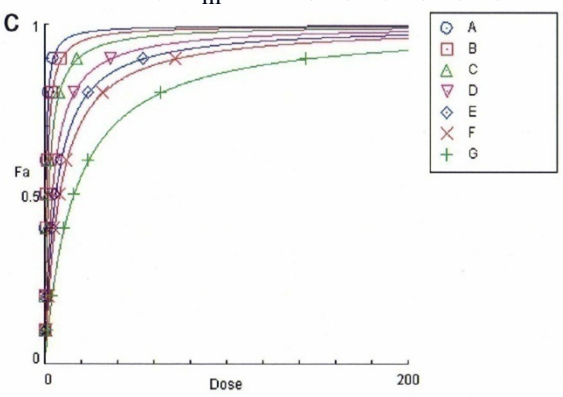
The Computer Simulation of the Median-Effect Equation

The Dose-Effect Curves: ***with different shape and potency***

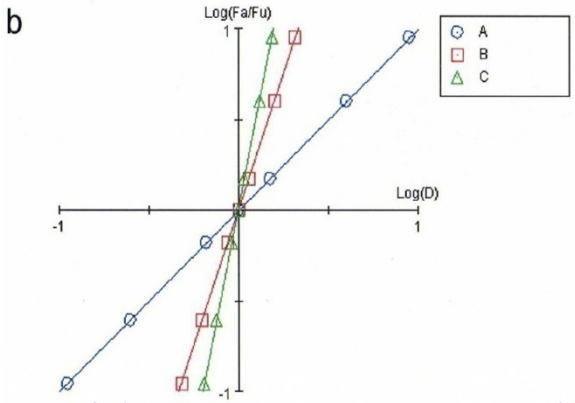
m = 1, 3, 5; D_m = 1

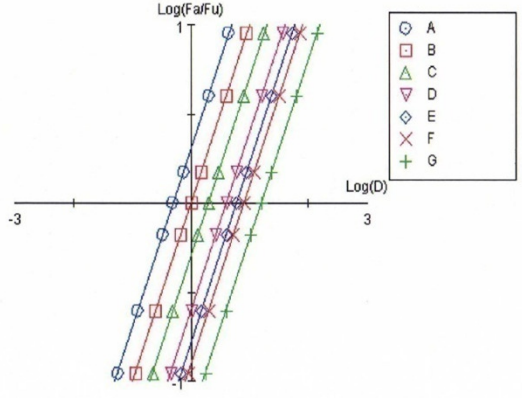


m = 1; D_m = 0.5, 1, 2, 4, 6, 8, 16



The Median-Effect Plot: (Chou Plot): ***The Linealization Principle***





The Revelation of A Fundamental New Concept: "The *Two Data-Point Theory*"

- **Dose-Effect Curves Follow the Median-Effect Principle of the Mass-Action Law The "Median" Serves as "The Universal Reference Point, and as The Common Denominator for Simplifying the Complicated Biological Systems". "One can draw a specific dose-effect curve with a *minimum* of "only two data points" --- When they are determined accurately. The Basis for "Econo-Green" Bio-Research.**

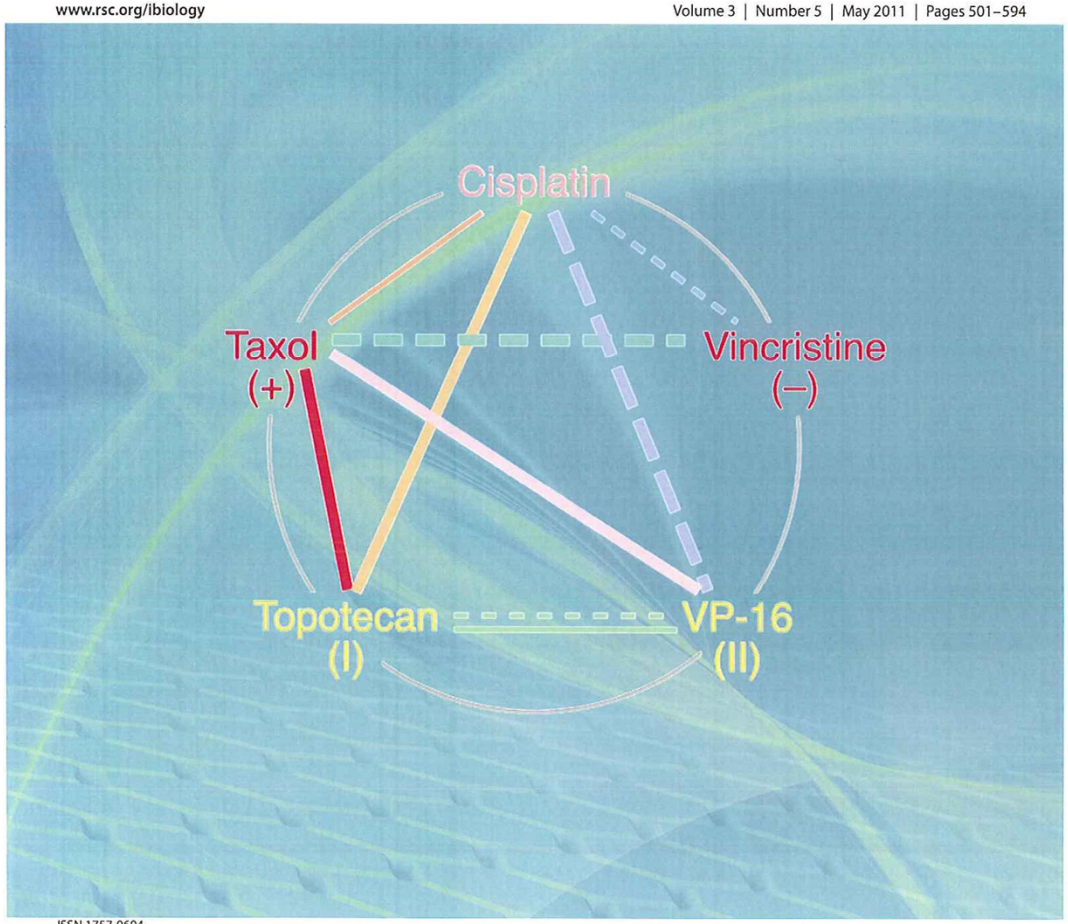
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Volume 3 | Number 5 | May 2011 | Pages 501–594



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PERSPECTIVE

Chou

The mass-action law based algorithms for quantitative econo-green bio-research

1757-9694(2011)3:5;1-S

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HOT: one equation to lead the way to greener biomedical sciences

05 May 2011

By Francesca Burgoyne



In this HOT Perspective article **Ting-Chao Chou** from the Memorial Sloan-Kettering Cancer Center, New York presents his vision for a new era of smarter, greener biomedical research and drug discovery.

The algorithm of the **median-effect equation** based on the **mass-action law,** along with experimental design and computer simulation, should allow a significant reduction in the number of data points required to yield useful bioinformatics on the relationship between dose and effect. He poses that a theoretical minimum of a mere two data points are required to construct dose-effect curves – if they are accurately determined. This unified theory, he believes, should pave the way for more efficient, cost-effective research and ethical clinical trials.

This HOT article received glowing reports from our referees and is featured on the front cover of our latest issue – **Issue 5**. Why not take a look – it's currently **free** to access:

The mass-action law based algorithms for quantitative econo-green bio-research

Ting-Chao Chou

Integr. Biol., 2011, **3**, 548-559 *[Published by Royal Society of Chemistry, Cambridge, UK]*

DOI: 10.1039/C0IB00130A 6177480

The Unified Theory: Dose & Effect Are "Interchangeable"

Derivation of Major Biochemical and Biophysical Equations from the Median-Effect Equation

[*Chou T.C. Pharmacol. Rev. 58: 621-681, 2006*]

Henderson-Hasselbalch equation

$$\log [H^+] = \log K_a + \log \frac{[HA]}{[A^-]}$$

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

Michaelis-Menten equation

$$v/V_{max} = [1+(K_m/S)]^{-1}$$

$$\frac{f_a}{f_u} = \left(\frac{D}{D_m}\right)^m$$

The Median-Effect Equation

Chou, J. Theor. Biol. 59: 253-276, 1976

$$f_a/(1-f_a) = (D/D_m)^m$$

$$f_a = [1+(D_m/D)^m]^{-1}$$

$$\log [(f_a/(1-f_a)] = m[\log(D) - \log D_m]$$

$$\log [(f_a)^{-1}-1]^{-1} = m \log(D) - m \log D_m$$

$$f_a/f_u = D/D_m$$

Hill equation

$$\log [v/(V_{max}-v)] = n \log(S) - \log (K)$$

Scatchard equation

$$\frac{[L]_b}{[L]_f} = \frac{n[M]_t}{K_d} - \frac{[L]_b}{K_d}$$

Algorithm for Computerized Simulation of Synergism, Additive Effect and Antagonism Based on the Combination Index Theorem for the Effects of Multiple Drugs

The Median Effect Equation

(1) $f_a/f_u = (D/D_m)^m$

(2) $\text{Log}(f_a/f_u) = m\log(D) - m\log(D_m)$

(3) $f_a = 1/[1+(D_m/D)^m]$

(4) $D_x = D_m[f_a/(1-f_a)]^{1/m}$

(5) $CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} = \frac{1}{(DRI)_1} + \frac{1}{(DRI)_2}$

$(D_x)_{1,2} = (D)_1 + (D)_2$

and $(D)_1/(D)_2 = P/Q$

$(D)_1 = (D_x)_{1,2} \times P/(P+Q)$

$(D)_2 = (D_x)_{1,2} \times Q/(P+Q)$

D = Dose

f_a = fraction affected

f_u = fraction unaffected

D_m = median-effect dose

m = slope, Hill-type coefficient or kinetic order

CI : Combination Index

CI = 1 (Additive Effect)

< 1 (Synergism)

> 1 (Antagonism)

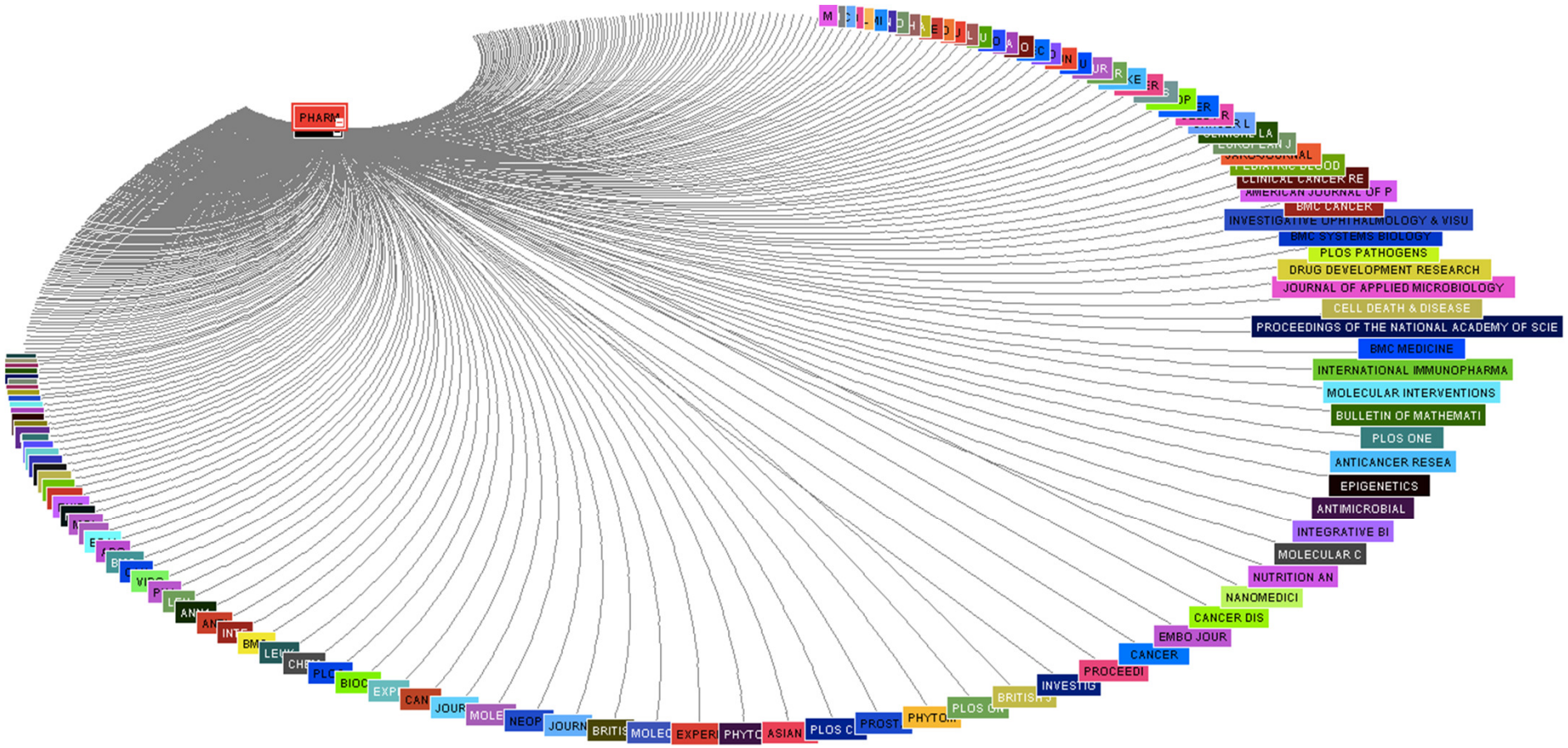
DRI: Dose-Reduction Index

$(DRI)_1 = \frac{(D_x)_1}{(D)_1}, \quad (DRI)_2 = \frac{(D_x)_2}{(D)_2}$

For *n* Drug Combination Index:

$$CI = \sum_{J=1}^{n} \frac{(D)_j}{(D_x)_j}$$

"Theoretical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies"



CHOU TC, PHARMACOLOGICAL REVIEWS, 58: 621-681, 2006

Times Cited: 1,408 (1,016 in 404 Journals)*

**[The Most Cited / the Broadest Cited Pharmacology Article in the World by Journals Since 2006]*

Effect of rIFNαA and AZT Singly or in Combination on RT (cpm/10^6 cells x 10^3) in Expt. 2, Day 10*

AZT (μM)	rIFNαA (*U*/ml) 0	8	16	32	64	128
0	205	173	150	193	169	151
0.01	159	85				
0.02	110		42			
0.04	71			10		
0.08	31				4	
0.16	7					1

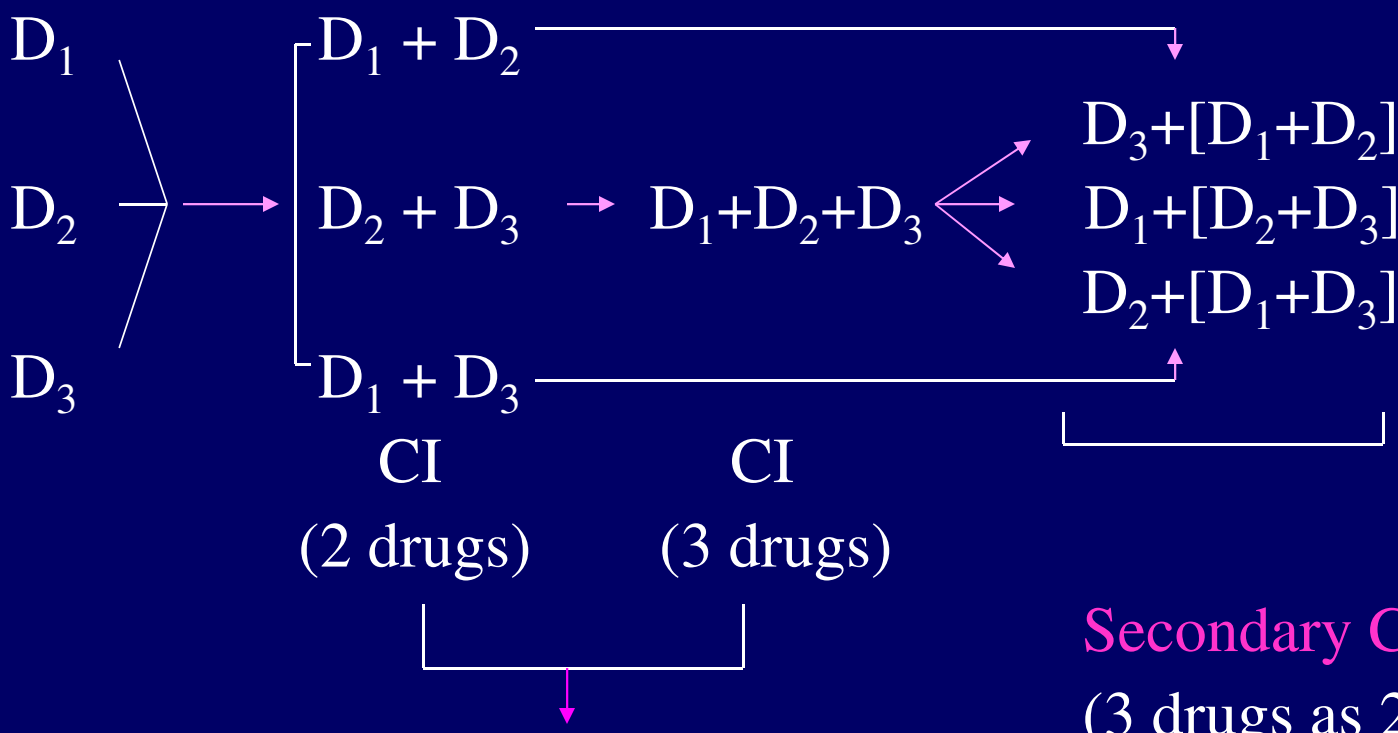
* From Hartshorn et al, Antimicrob. Agents Chemother. 31: 168-172, 1987

(MGH, Harvard Medical School/Chou, MSKCC) (Cited by 252 articles)

Constant Ratio Design Based on IC_{50} Ratios

D_1	+	D_2	+	D_3
a	:	b	:	c
a	:	b		
		b	:	c
a		:		c

Dissection of Three Drug Combination



Primary CI

Are results of 3D combination predictable from 2D combinations ?

Secondary CI

(3 drugs as 2 drugs)

How the 3rd drug affects the 2D combinations ?

CompuSyn

For Single Drug and Drug Combinations

A Computer Program for Quantitation of Synergism and Antagonism in Drug Combinations, and the Determination of IC_{50}, ED_{50} and LD_{50} Values.

By Ting-Chao Chou (MSKCC)

and Nick Martin (MIT)

Published and Distributed by ***ComboSyn, Inc.***

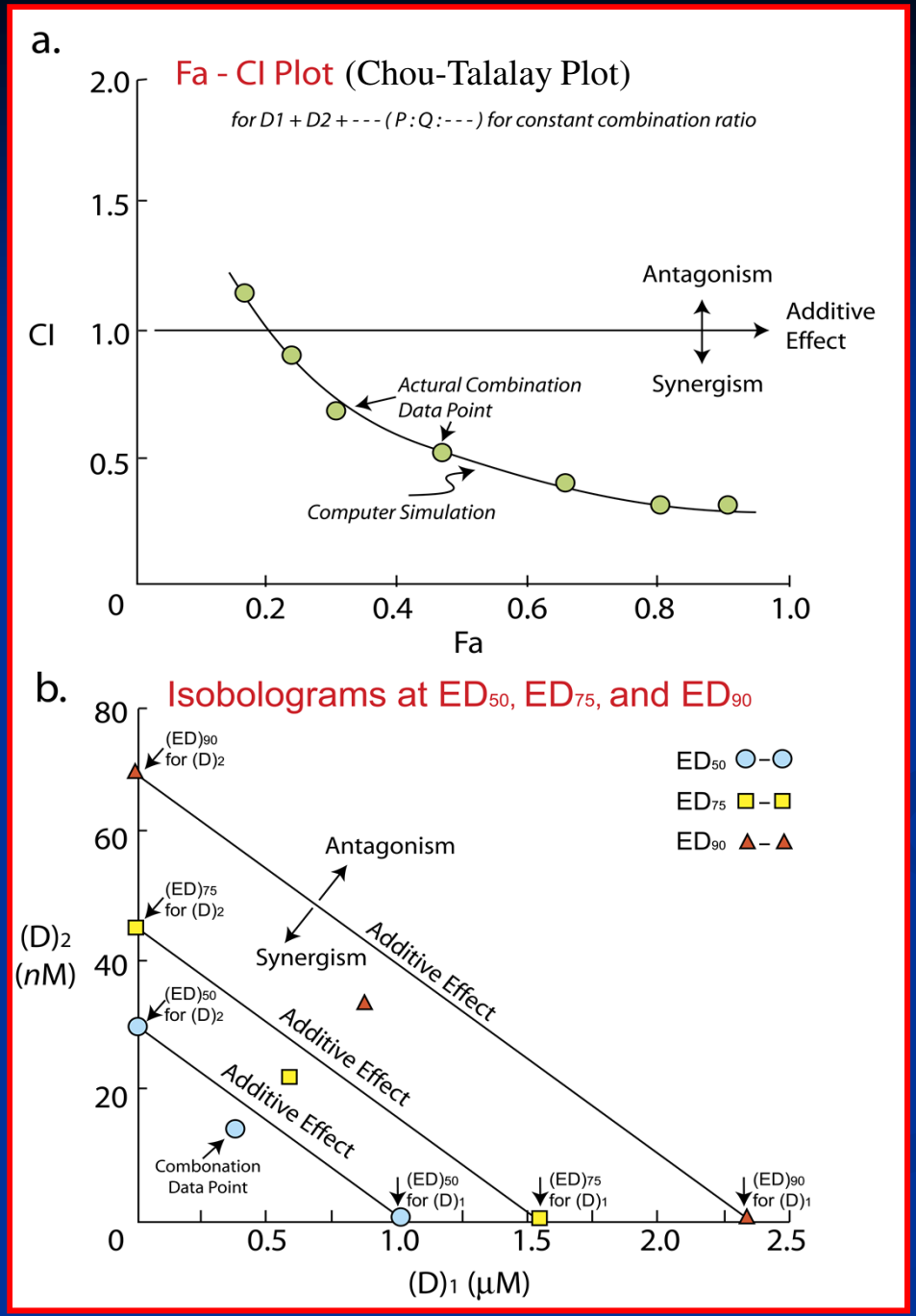
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– PD Science, LLC



Diagnostic Plots

Fa-CI Plot: Effect-Oriented

[Combination Index, CI]

CI < 1: Synergism

CI = 1: Additive

CI > 1: Antagonism

Isobologram: Dose- Oriented

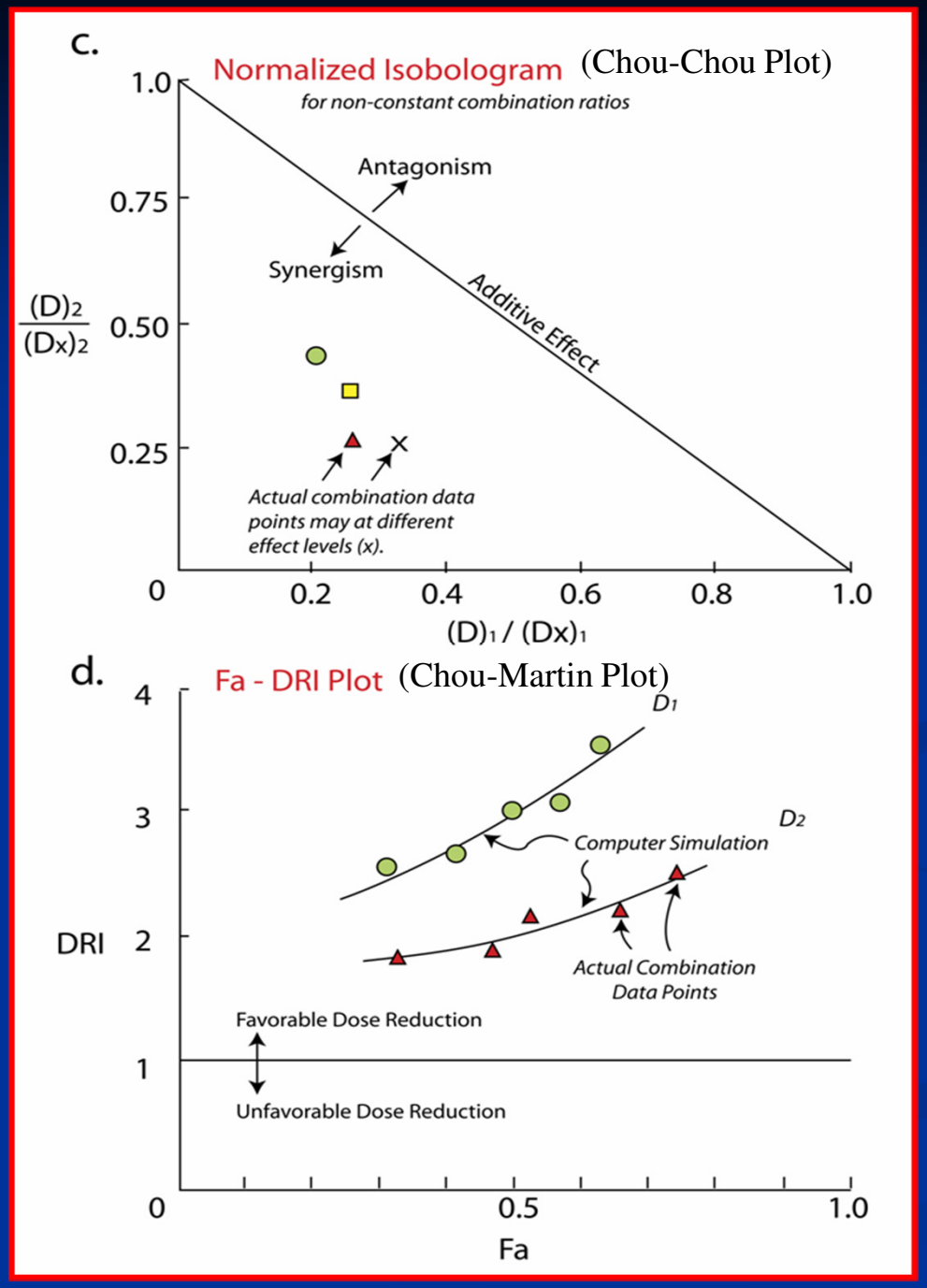
Points on Line: Additive

Points on Lower Left: Synergism

Points on the Upper Right: Antagonism

Both are Two-sides of the Same Coin *[Isobol Eq. derived by Chou in 1983]*

[Chou TC, Pharmacol. Rev. 58:621-681, 2006]

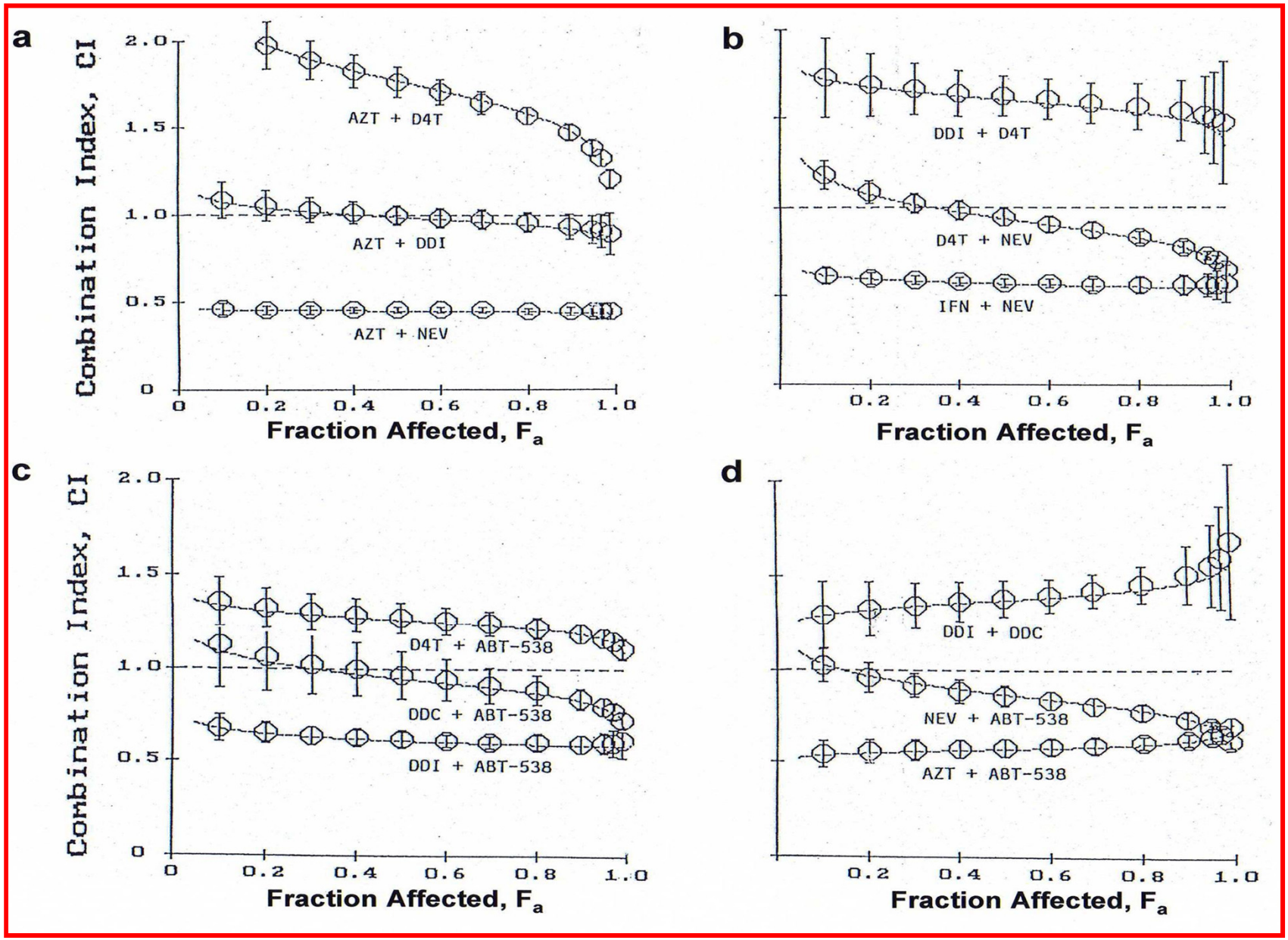


Diagnostic Plots

Dose Normalized Isobol (ChouTC & Chou J, 1983, software)

Dose Reduction Index (Chou TC & Chou J, 1988, equation)

DRI = 1
No dose reduction
DRI > 1 Favorable Dose Reduction
DRI < 1 Not Favorable Dose Reduction


a
Combination Index, CI
2.0
1.5
1.0
0.5
0
AZT + D4T
AZT + DDI
AZT + NEV
0
0.2
0.4
0.6
0.8
1.0
Fraction Affected, Fa
b
DDI + D4T
D4T + NEV
IFN + NEV
0.2
0.4
0.6
0.8
1.0
Fraction Affected, Fa
c
Combination Index, CI
2.0
1.5
1.0
0.5
0
D4T + ABT-538
DDC + ABT-538
DDI + ABT-538
0
0.2
0.4
0.6
0.8
1.0
Fraction Affected, Fa
d
DDI + DDC
NEV + ABT-538
AZT + ABT-538
0.2
0.4
0.6
0.8
1.0
Fraction Affected, Fa

Typical example of changes in DRI values and their relationship to CI values in two to five drug combinations (Chou-Talalay Method)

Drug Combinations	DRI[a] values at				CI[b] values at			
	EC_{50}	EC_{75}	EC_{90}	EC_{95}	EC_{50}	EC_{75}	EC_{90}	EC_{95}
AZT +	3.87	3.85	3.83	3.82	0.455	0.451	0.446	0.444
NEV (1:40)	5.09	5.24	5.40	5.50				
AZT +	5.43	5.12	4.89	4.74				
NEV +	7.08	6.97	6.86	6.79	**0.368**	**0.371**	**0.377**	**0.381**
IFN (1:40:16)	24.36	30.76	38.90	45.57				
AZT +	3.80	4.14	4.33	4.56				
NEV +	5.06	5.57	6.14	6.55	0.602	0.574	0.548	0.532
ABT-538 (1:40:4)	6.87	6.61	6.38	6.20				
AZT +	6.0	6.0	6.0	6.05				
NEV +	7.81	8.16	8.52	8.79	**0.426**	**0.420**	**0.418**	**0.416**
ABT-538 +	10.61	9.68	8.87	8.33				
IFN (1:40:4:16)	26.89	36.00	48.26	58.93				
AZT +	5.18	5.80	6.29	6.62				
NEV +	6.99	7.84	8.79	9.51				
ABT-538 +	9.50	9.28	9.13	9.00	**0.609**	**0.539**	**0.485**	**0.455**
IFN +	23.95	34.65	49.83	63.78				
DDI + (1:40:4:16:1600)	7.90	9.77	12.09	13.97				

[a] Dose reduction index (DRI) provides a measure of how much (fold) the dose of each drug in a synergistic combination may be reduced at a given effect level compared with the doses of each drug alone. The relationship of CI and DRI is depicted in Eq. 16.

[b] CI values are obtained from Tables 22 and 23 of Pharmacol.Rev. 58:621-681,2006..

Quantitative *vs* Semi-quantitative Informatics

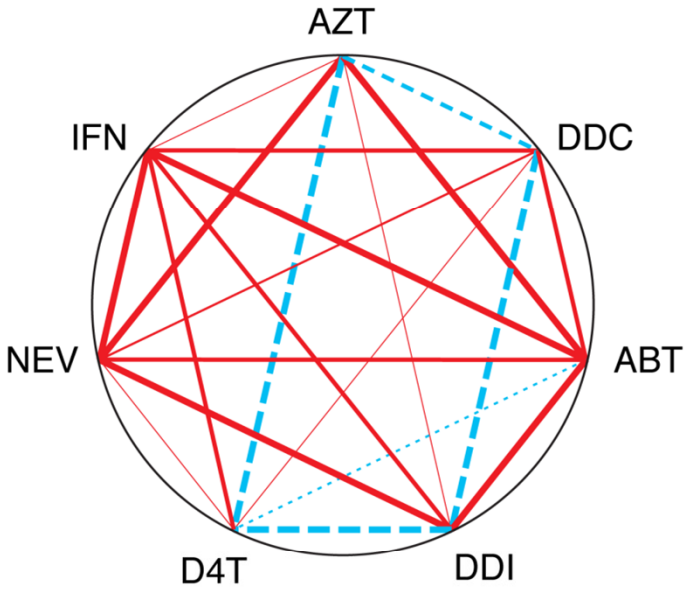
How Much Synergy Is Synergism?

[Chou TC. Pharmacol Rev 58:621-681, 2006]

Range of combination index	Description	Graded symbols	Graphic symbols
<0.1	very strong synergism	+++++	
0.1-0.3	strong synergism	++++	
0.3-0.7	synergism	+++	
0.7-0.85	moderate synergism	++	
0.85-0.90	slight synergism	+	
0.90-1.10	nearly additive	±	
1.10-1.20	slighht antagonism	−	
1.20-1.45	moderate antagonism	− −	
1.45-3.3	antagonism	− − −	
3.3-10	strong antagonism	− − − −	
>10	very strong antagonism	− − − − −	

[a] Combination index method is based on those described by Chou and Talalay (1984) and the computer software of Chou and Martin (2005). The ranges of CI and the symbols are refined from those described earlier by Chou (1991). CI<1, =1, and >1 indicates synergism, additive effect and antagonism, respectively. (Modified from Chou and Martin, 2005)

a. Two Drug Combinations for Seven Anti-HIV Agents and the Heptagonal Polygonograms

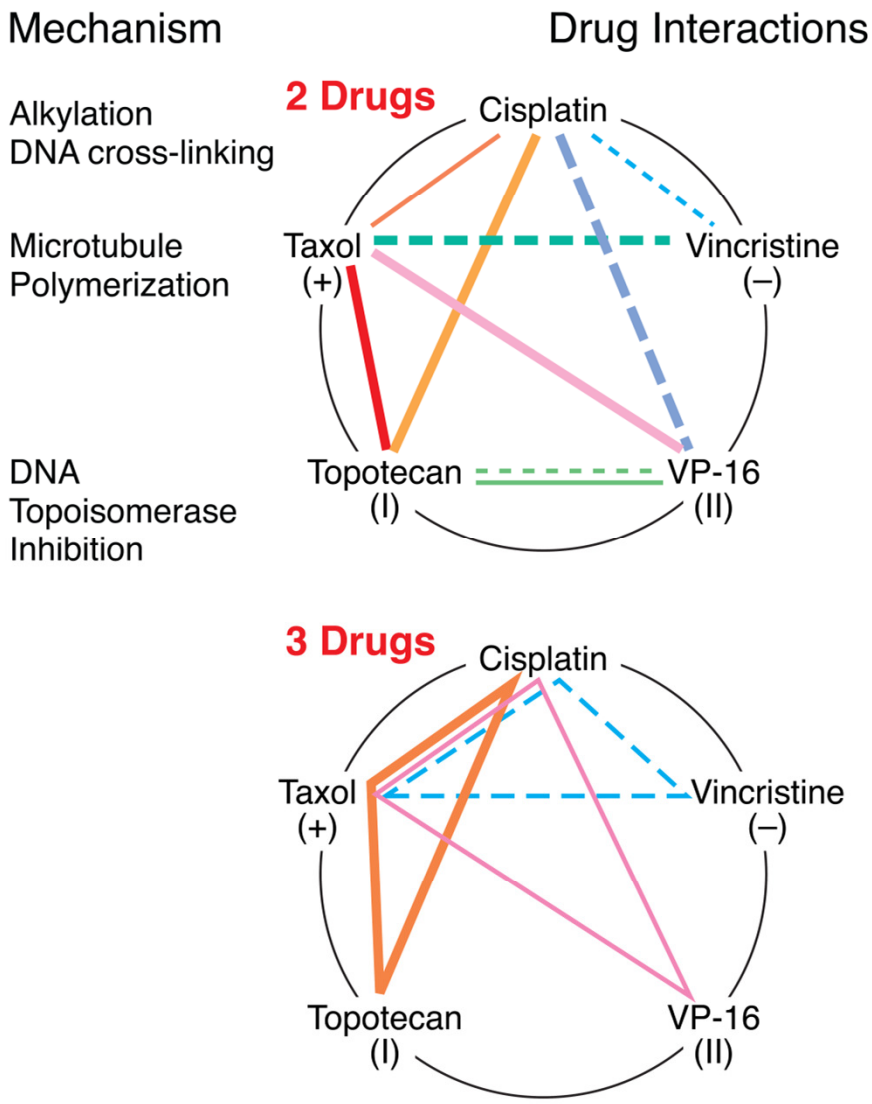


Synergism +++
Moderate Synergism ++
Slight Synergism +
Nearly Additive Effect ±

Antagonism – – –
Moderate Antagonism – –
Slight Antagonism –

$$\sum_{r=2}^{7} C_r^7 = 120$$

b. Polygonograms for Five Anticancer Agents with Different Mechanisms of Action



General Equation for 5-Drug Combination

(Chou TC, Pharmacol. Rev. 58: 621-681, 2006)

$$
{}^{5}(CI)_x = \frac{(D_x)_{1\text{-}5}[P/(P+Q+R+S+T)]}{(D_m)_1\left\{(fa_x)_1/[1-(fa_x)_1]\right\}^{1/m_1}} + \frac{(D_x)_{1\text{-}5}[Q/(P+Q+R+S+T)]}{(D_m)_2\left\{(fa_x)_2/[1-(fa_x)_2]\right\}^{1/m_2}}
$$

$$
+ \frac{(D_x)_{1\text{-}5}[R/(P+Q+R+S+T)]}{(D_m)_3\left\{(fa_x)_3/[1-(fa_x)_3]\right\}^{1/m_3}} + \frac{(D_x)_{1\text{-}5}[S/(P+Q+R+S+T)]}{(D_m)_4\left\{(fa_x)_4/[1-(fa_x)_4]\right\}^{1/m_4}}
$$

$$
+ \frac{(D_x)_{1\text{-}5}[T/(P+Q+R+S+T)]}{(D_m)_5\left\{(fa_x)_5/[1-(fa_x)_5]\right\}^{1/m_5}}
$$

General Equation for N-Drug Combination

(Chou TC. Pharmacol. Rev. 58:621-681, 2006)

$$ {}^{n}(CI)_x = \sum_{i=1}^{n} \frac{(D_x)_{1\sim n}\left\{[D]_i \Big/ \sum_{1}^{n}[D]\right\}}{(D_m)_i\left\{(fa_x)_i \Big/ [1-(fa_x)_i]\right\}^{\frac{1}{m_i}}} $$

${}^{n}(CI)_x$ Combination index for n drugs at x% inhibition

$(D_x)_{1\sim n}$ The sum of n drugs that exerts x% inhibition in combination

$\left\{[D]_i \Big/ \sum_{1}^{n}[D]\right\}$ The proportionality of each of n drugs that exerts x% inhibition in combination

$(D_m)_i\left\{(fa_x)_i \Big/ [1-(fa_x)_i]\right\}^{\frac{1}{m_i}}$ The dose of each drug alone that exerts x% inhibition.

Where D_m: The median-effect dose (Antilog of the x-intercept of the median-effect plot)

fa_x: Fractional inhibition at x% inhibition

m: The slope of the median-effect plot which depicts the shape of the dose-effect curve. m=1, >1, and <1 indicates, hyperbolic, sigmoidal, and negative sigmoidal curve, respectively.

Computerized Simulation of Synergism/Antagonism

[The Econo-Green & Quantitative Bio-Informatics]

Primary Questions:

- Are there any synergism?
- How much synergism?
- Synergism at what dose levels?
- Synergism at what effect levels?
- What the exhibited isobologram looks like?
- How many folds dose reduction for each drug as results of synergism?

Other Questions:

- Optimal combination ratio
- Schedule dependency
- Selectivity of synergism
- Condition directed synergism



Thanks

More Details will be given on Day3 Symposium, Wednesday 9-10 AM

CI<1 (Synergism); CI=1 (Additive); CI >1 (Antagonism). What, When, Why, How, Where